A STUDY OF SOME FACTORS INFLUENCING THE STIMULATIVE ACTION OF ZINC SULPHATE ON THE GROWTH OF ASPERGILLUS NIGER. I. THE EFFECT OF THE PRESENCE OF ZINC IN THE CULTURAL FLASKS

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The increased growth of *Aspergillus niger* resulting from the introduction of zinc salts into the nutrient solution was first observed by Raulin (9). This observation was confirmed by Richards (10) in 1897, who noted also that other elements (Co, Ni, F, Fe, etc.) exercised an influence similar to that of zinc.

The studies of these two investigators, as well as those of Ono (8), of Richter (11), and of others agree in that there is the greater growth in the presence of zinc, although the percentage increase the various authors obtained is not identical.

In 1903, however, a publication by Coupin (2) appeared, in which this author came to the conclusion that the increased dry weights formed in cultures of *Aspergillus niger* through the addition of zinc salts takes place only in impure cultures. The addition of zinc is effective, in this investigator's opinion, only in those cases in which there is an opportunity for the suppression of the activities of other organisms, whose presence in the *Aspergillus niger* cultures prevents the full development of this fungus. In pure cultures the maximum dry weight is obtained without the addition of zinc.

Another interpretation of Coupin's results has been suggested by Javillier (3)—namely, the introduction of zinc into the nutrient liquid through solution of constituents of the glass of the cultural flask. Javillier, indeed, states that with cultures in Jena glass (flasks of the same kind of glass were made use of by Coupin) the addition of zinc is unnecessary inasmuch as the maximum growth

(about 1 gram per 50 c.c. nutrient solution) takes place. Simultaneous cultures in Kavalier Bohemian and quartz flasks did not exceed a yield of about 0.3 grams per 50 c.c. nutrient solution unless zinc was added.

According to Lepierre (5, 6), on the other hand, the increased yields obtained by Coupin should be ascribed to the excessive volume of nutrient solution employed as compared to the volume of the containing flask. The increased depth of the solution, he assumes, results in decreased aëration of the cultures and increased growth ensues. Zinc, it is stated by Lepierre (6), could be found neither in the flasks (whether Jena or Kavalier is not stated) or compounds used nor in the fungal membranes.

Javillier (4), in repeating Lepierre's experiments in Kavalier glass, did not succeed in obtaining an essential variation in the yield by varying the ratio of surface to volume.

It is interesting to note in this connection that the presence of zinc in Jena glass has frequently been reported in the literature, more recently through the analyses of Nicolardot (7) and of Walker and Smither (12). Moreover, Kavalier Bohemian glass according to the same authors is free from zinc.

That the composition of the cultural flasks is of importance and that both the rate of growth and the fructification of *Aspergillus niger* can be influenced by solution of components of the glass has been claimed by Benecke (1).

To obtain, if possible, additional evidence as to whether cultures of *A. niger* to which no zinc has been intentionally added attain a greater growth in Jena glass than in Kavalier Bohemian, I have grown parallel cultures in these two glasses, and in addition, a third glass, Pyrex. Zinc according to Walker and Smither (12) does not enter into the composition of Pyrex glass.

In the experiments whose description follows the Pfeffer nutrient solution has been used (10).

PFEFFER SOLUTION

	Grams
Water	1000.0
Cane sugar	50.0
Ammonium nitrate	10.0
Mono-potassium phosphate	5.0
Magnesium sulphate	2.5
Iron sulphate	trace

The compounds employed in the preparation of this solution were water redistilled through glass; Merck's "Reagent" ammonium nitrate and magnesium sulphate; Kahlbaum's "Zur anal." magnesium sulphate; and Baker's "Analyzed" potassium phosphate, ferric sulphate, and zinc sulphate. The cane sugar used is that sold under the proprietary name of "Crystal Domino" sugar. This solution was prepared as needed.

The flasks used were: 200 c.c. Jena and 150 c.c. Pyrex Erlenmeyer's; and 250 c.c. Kavalier Bohemian Florence flasks. They were cleaned by rinsing with concentrated sulphuric acid, tap-water, lastly distilled water, and inverted to drain dry. The precaution was taken of reserving part of the flasks for zinc-free cultures and part for zinc cultures only, though this performance is unnecessary, as the following indicates:

1. ON THE EFFICIENCY OF THE METHOD FOR CLEANING THE CULTURAL FLASKS

Pfeffer solution: water redistilled through glass; "Crystal Domino" sucrose; NH_4NO_3 (Merck); KH_2PO_4 (Baker); $MgSO_4 \cdot 7H_2O$ (Kahlbaum). Temperature 30–31° C. Period of growth 7 days. No zinc added. Pyrex flasks.

Flasks reserved for zinc-free cultures	Flasks reserved for zinc cultures
0.924 grams*	0.283 grams
0.336 "	0.265 "
0.321 "	0.313 "
0.328 "	0.341 "
0.355 "	0.352 "
0.335 "	0.311 "

The flasks in the second column were previous to this experiment used three consecutive times for cultures containing 10 mg. Zn/L. and were cleaned as usual.

Zinc was added where indicated to the entire solution used in the preparation of cultures having the same concentration of this heavy metal and not to the individual flasks. The stock solu-

* High yield, due probably to the accidental introduction of zinc. This value not included in the average. In at least one case it was noted that the same Pyrex flask consistently gave cultures having a high yield, though the addition of zinc was omitted.

tion contained 2.5 mg. zinc per cubic centimeter (i. e., 11.0 mg. $ZnSO_47.H_2O$ per c.c.).

The flasks, each containing 50 c.c. of nutrient solution measured in a 50 c.c. graduate, were sterilized at 14½ lbs. for 20 minutes.

Inoculations were from stock bread-cultures grown at room temperature (18–25° C.). Enough spores were added with a platinum loop to make a visible and apparently almost continuous layer on the solution surface. The *A. niger* culture used in these experiments was obtained originally from the "Internationalstelle für Pilz-Kulturen, Amsterdam." Immediately after inoculation the flasks were placed in a dark cupboard at room temperature (18–23° C.), or in the thermostat at 30–31 ° C.

When harvested, the membrane, together with the solution, was thrown on a washed and weighed filter, washed with distilled water and dried at 103–105° C. for four days.

The yields while given to the third place are probably reliable to two places only.

2. THE EFFECT OF ADDING ZINC TO THE CULTURE MEDIUM

Pfeffer solution: water redistilled through glass; "Crystal Domino" sucrose; NH_4NO_3 (Merck); KH_2PO_4 (Baker); $MgSO_4.7H_2O$ (Merck). Room temperature (18–23° C.). Period of growth ten days.

Kavalier Bohemian		Pyrex	
No zinc	10 mg. Zn/L	No zinc	10 mg. Zn/L
0.317 grams	0.888 grams	0.387 grams	0.864 grams
0.330 "	0.905 "	0.328 "	0.855 "
0.341 "	0.886 "	0.306 "	0.878 "
0.302 "	0.880 "	0.286 "	0.868 "
0.314 "	0.924 "	0.325 "	0.903 "
0.321 "	0.897 "	0.326 "	0.874 "

Here we see that in the Kavalier and Pyrex flasks the addition of zinc to the nutrient solution results in an increased dry weight, the increase being almost three-fold.

3. THE INFLUENCE OF A ZINC GLASS (JENA) ON THE YIELD

Pfeffer solution: water redistilled through glass; "Crystal Domino" sucrose; NH_4NO_3 (Merck); KH_2PO_4 (Baker); $MgSO_4.$-

$7H_2O$ (Kahlbaum). Room temperature (18–23° C.). Period of growth ten days.

Jena		Kavalier Bohemian		Pyrex	
No zinc	10 mg. Zn/L	No zinc	10 mg. Zn/L	No zinc	10 mg. Zn/L
0.989 gr.	0.980 gr.	0.270 gr.	0.924 gr.	0.319 gr.	0.940 gr.
0.958 "	0.940 "	0.299 "	0.943 "	0.248 "	0.980 "
0.919 "	1.005 "	0.285 "	0.886 "	0.306 "	0.917 "
0.933 "	0.988 "	0.300 "	0.947 "	0.252 "	0.952 "
0.953 "	1.022 "	0.351 "	1.017 "	0.309 "	0.997 "
0.950 "	0.987 "	0.301 "	0.943 "	0.287 "	0.957 "

With nutrient solutions to which no zinc has been added a distinct difference is displayed, as concerns the yield, between the Jena flasks, on the one hand, and the Kavalier and Pyrex on the other. The low yields characteristic of the zinc-free culture are obtained only in the latter two glasses.

Cultures grown in the presence of 10 mg. zinc (calculated as metal) per liter attained a dry weight of approximately one gram irrespective of the kind of flask used.

Additional experiments bringing out the increased growth taking place in a culture medium to which no zinc has been intentionally added when in Jena glass are as follows:

4. THE INFLUENCE OF A ZINC GLASS (JENA) ON THE YIELD

Pfeffer solution: water redistilled through glass; "Crystal Domino" sucrose; NH_4NO_3 (Merck); KH_2PO_4 (Baker); $MgSO_4 \cdot 7H_2O$ (Kahlbaum). Temperature 30–31° C. Period of growth seven days. No zinc added.

Pyrex	Jena
0.247 grams	0.948 grams
0.232 "	0.896 "
0.327 "	0.893 "
0.279 "	0.904 "
0.257 "	0.904 "
0.268 "	0.909 "

5. CONDITIONS AS IN THE PRECEDING EXPERIMENT

Pyrex	Jena
0.336 grams	0.915 grams
0.316 "	0.849 "
0.376 "	0.881 "
0.332 "	0.872 "
0.296 "	0.864 "
0.331 "	0.876 "

Here also we see that while addition of zinc is necessary to bring about increased growth in Pyrex flasks, this increase in growth occurs in the cultures in Jena glass to which no zinc has been intentionally added.

A comparison of the values obtained in these experiments will show the agreement in the dry weights of individual duplicate cultures, and of the mean values (average of five duplicate cultures) obtained in the different experiments. On the average the deviation from the mean does not exceed 0.040–0.050 gram, in exceptional instances deviations of as much as 0.10–0.15 gram being encountered. This means, therefore, a variation of about 5 per cent for the zinc cultures and of 15 per cent for the zinc-free cultures, since the mean weight of the former is about 0.95 grams, of the latter 0.30 grams.

We are therefore justified in concluding that while the addition of zinc serves to bring about an increased formation of dry weight in Kavalier Bohemian and Pyrex flasks, such increased formation of dry weight takes place in Jena flasks to which no zinc is intentionally added. That the increased growth that occurs in the Jena flasks is due to the solution of small amounts of zinc from the flasks is also highly probable in view of the presence of zinc in this glass.

LIST OF PAPERS REFERRED TO IN TEXT

1. **Benecke, W.** Die Bedeutung des Kaliums und des Magnesiums für Entwickelung und Wachstum des *Aspergillus niger* v. Th. sowie einiger anderer Pilzformen. Bot. Zeit. **54**: 97. 1896.
2. **Coupin, H.** Sur la nutrition du *Sterigmatocystis nigra*. Compt. Rend. **136**: 392. 1903.
3. **Javillier, M.** Une cause d'erreur dans l'étude de l'action biologique des éléments chimiques: la présence de traces de zinc dans le verre. Compt. Rend. **158**: 140. 1914.
4. **Javillier, M.** Utilité du zinc pour le développement de l'*Aspergillus niger* cultivé sur milieux profonds. Bull. Soc. Chim. IV. **15–16**: 568. 1914.
5. **Lepierre, C.** Inutilité du zinc pour la culture de l'*Aspergillus niger*. Compt. Rend. **157**: 876. 1913.
6. **Lepierre, C.** Zinc et *Aspergillus*. Les expériences de M. Coupin et de M. Javillier. Compt. Rend. **158**: 66. 1914.

7. **Nicolardot, P.** Sur l'attaque des verres de France, de Bohême et d'Allemagne. Compt. rend. **163**: 355. 1916.
8. **Ōno, N.** Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sci. Imp. Univ. Tokyo **13**: 142. 1900.
9. **Raulin, J.** Études chimique sur la végétation. Ann. Sci. Nat. Bot. V. **11**: 93. 1869.
10. **Richards, H. M.** Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**: 665. 1897.
11. **Richter, A.** Zur Frage der chemischen Reizmittel. Die Rolle des Zn und Cu bei der Ernährung von *Aspergillus niger*. Centralbl. Bakt. II. 7: 417. 1901.
12. **Walker, P. H. & Smither, F. W.** Comparative tests of chemical glassware. Journ. Ind. & Eng. Chem. **9**: 1090. 1917.